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Transdermal and Topical Drug Delivery Systems

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1997

Interpharm Press, Inc.
Buffalo Grove, Illinois

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SKIN ABSORPTION ENHANCEMENT BY PHYSICAL MEANS: HEAT, ULTRASOUND, AND ELECTRICITY

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There have been a number of comprehensive reviews on the subjects of drug absorption enhancement through the skin by iontophoresis (Harris 1967; Sloan and Soltani 1986; Tyle 1986; Chien et al. 1988; Singh and Roberts 1989; Burnette 1989) and phonophoresis (Griffin 1982; Skauen and Zentner 1985; Tyle and Agrawala 1989; Ghosh and Banga 1993; Kost 1993). The objective of this chapter is to selectively discuss some of the important aspects of physical means of enhancing drug permeation through the skin. This approach may help to provide additional information on certain less-noticed areas, such as the effect of thermal energy on percutaneous drug absorption and its potential applications. Some recent advances in the use of electric and acoustic energies for transdermal drug delivery (TDD) will also be highlighted. An attempt will be made to link the basic principles involved with experimental evidences in order to help the reader better understand and fully utilize these useful modalities.

CHEMICAL PHYSICAL DRUG PERMEATION ENHANCEMENT

One of the primary functions of human skin is to act as a barrier, to prevent the body from losing water into the environment and to block the entry of

any exogenous chemicals into the body. In this sense, it is not so "natural" to administer a drug through intact skin to achieve either a local (topical) or a systemic (transdermal) therapeutic effect. The barrier function is attributed primarily to the outermost layer of keratinized dead cells of the stratum corneum (Scheuplein and Blank 1971). In order to transport a drug across the skin barrier, one needs to rely on at least one of several energy forms.

The energy form almost always involved in skin permeation is the chemical potential of a transported drug. The chemical potential of a substance of interest is defined in thermodynamics as the partial molar free energy of the substance. The difference between the chemical potentials of a drug outside and inside the skin is the energy source for the skin permeation process. Since the chemical potential of a drug is closely associated with its concentration, we may regard the drug concentration gradient across the skin barrier as the driving force for skin permeation by passive diffusion. Drug molecules or ions diffuse through the stratum corneum, driven by the higher drug concentration on the skin surface, to reach the targeted sites in the viable epidermis and dermis, and are absorbed by the dermal microvasculature into the systemic blood circulation. The factors influencing the simple passive diffusion process are the applied drug concentration; the size of the drug molecule, which defines the drug diffusivity in various layers of the skin, most importantly, in the stratum corneum; and the affinity of the drug toward the lipophilic stratum corneum, usually expressed as the partition coefficient of the drug between an oily phase and an aqueous phase. Fick's first law for steady state diffusion states (Martin et al. 1983):

$$J = \frac{dM}{Adt} = \frac{DK(C_d - C_r)}{h} \qquad \text{(eq. 1)}$$

in which J is the flux, M is the amount of a permeant transporting through a cross-section area, A, of a membrane barrier in time t, D is the diffusion coefficient, K is the partition coefficient, C_d is the permeant concentration at the donor side or the upstream of the diffusion process, C_r is the permeant concentration at the receptor side or the downstream, and h is the thickness of the membrane.

Because human skin, more specifically, the stratum corneum, is such an effective barrier, only a very small number of drugs can easily penetrate through in therapeutic quantities. Nitroglycerin, scopolamine, hydrocortisone, betamethasone, benzocaine, and lidocaine are a few examples. A great deal of research has been conducted during the past two decades searching for means to enhance drug penetration into the skin. As shown in Equation 1, the flux of drug permeation through the skin (J) can be increased by varying any of the three parameters on the right-hand side of the equation (i.e., the difference in the drug concentrations at both sides of the skin

$[C_d - C_r]$ the drug diffusion coefficient $[D]$, and partition coefficient $[K]$ in the skin). The thickness of the stratum corneum is regarded as an intrinsic skin property depending only on the anatomical site and the type of skin, and, therefore, is often treated as a constant. It is now known that many chemicals can influence the drug diffusion coefficient and partition coefficient to increase skin permeation. They are called skin permeation enhancers. The topic of chemical skin permeation enhancers is discussed extensively elsewhere in this book.

The other energy forms often employed to facilitate drug transport across the skin barrier are thermal energy, ultrasound, and electricity. Unlike chemical permeation enhancers, these permeation enhancing methods are of a physical nature. Equation 1 is applicable to the thermal method, and to some extent to the ultrasound method. In electrically facilitated skin permeation, the dominant driving force is from the applied electric potential gradient across the skin, which is added to the chemical potential gradient of a permeant. Depending on the mechanisms of drug transport, which is largely determined by the mode of the applied electric voltage and current, the method of electrically facilitated drug transport through the skin barrier may be further divided into transdermal iontophoresis, electro-osmosis, and electroporation. Transdermal iontophoresis is characterized by ionic drug migration into the skin driven by an applied electric potential gradient. Electro-osmosis is described as the transport of drug species, often nonionic, carried by the movement of the medium. The electro-osmotic flow of the medium is also driven by the applied electric potential gradient. Electro-osmosis can be used to deliver nonionic drug species into the skin. Conversely, it can also be used to transport biosubstances out of the skin for monitoring purposes. Electroporation describes the microscopic perforation of the skin barrier by extremely short pulses of high electric voltage and low current. In most cases, a physical, permeation-enhancing method operates along with the drug concentration gradient to facilitate the kinetic process of drug transport, although transdermal iontophoresis and electro-osmosis are capable of, at least in theory, delivering drug into the skin without the assistance of the permeant concentration gradient.

PERMEATION ENHANCEMENT BY THERMAL ENERGY

Temperature Effects on Drug Diffusion

The use of heat to improve topical drug adsorption is a time-honored practice. Heat is a form of energy that passes from one body to another due to a temperature difference between them. In thermodynamic terms, heat is the internal energy that a body possesses. High temperature means an elevated

internal energy, which implies accelerated thermal movement of particles, resulting in an increased drug diffusion coefficient. The high temperature of a system also influences the drug concentration gradient by improving the slow kinetic processes of drug dissolution and by increasing drug solubility in the donor solution (C_d).

Heat has an effect on the human sensory system and has a direct impact on dermal blood circulation. The skin responds to applied heat or elevated environmental temperature by dilating blood vessels and increasing blood flow (Lehmann and De Lateur 1990a), which accelerates the removal of drug in the skin (i.e., reducing C_r in Equation 1), thus leading to increased drug permeation.

Skin Temperature Site Variations

Skin temperature is influenced by many factors, including anatomical site, age, certain disease conditions, clothing, environment temperature, and so on. Figure 10.1 shows the distribution of tissue temperature in the human body after exposure to cold and heat (Wenger and Hardy 1990). Using a thermal imaging technique, Ring (1995) obtained the distribution of skin temperature of a male subject after 20 minutes in ambient temperature. It was found out, even under a relatively normal environmental temperature, that the skin temperature may vary from 24°C to 36°C depending on the anatomical location. Under more extreme environmental conditions, such as direct exposure to the wind in the winter and the sun in summer, a much greater variation of the skin temperature is certainly expected.

Effect of Temperature on In Vitro Drug Permeation

The effect of temperature on membrane permeation of drugs under asymmetric temperature conditions was investigated to mimic constant body temperature and varied environmental temperature (Tojo et al. 1987). Desoxycorticosterone and testosterone were used as the model permeants and silicone membrane as the model barrier membrane. Saturated drug suspensions were used as the donor solution. The in vitro permeation study was set up in such a way that while the receptor chamber temperature was maintained constant at 37°C, the donor chamber temperature varied from 10, 20, 30, 37, 50, and 60°C in each permeation experiment. The permeation results are shown in Figures 10.2a and 10.2b. As expected, drug permeation was directly related to donor temperature. It was found that using the membrane temperature (the average temperature of the donor and receptor chambers) as the system temperature, the Arrhenius relationship holds for the drug

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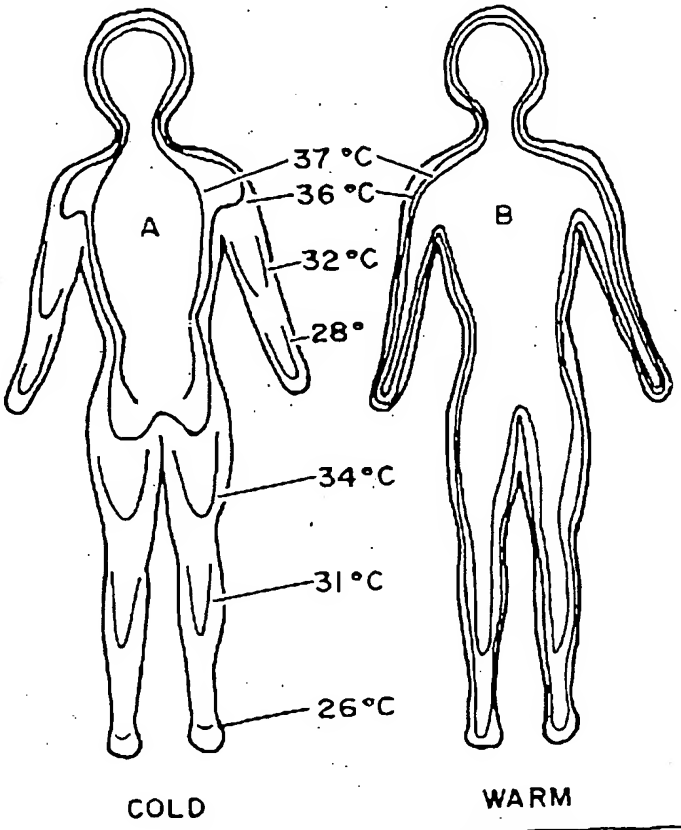
Figure 10.1. Distribution of Tissue Temperature and Isotherms in the Body



A: After exposure to cold. B: After exposure to heat. Reproduced with permission from Wenger and Hardy (1990).

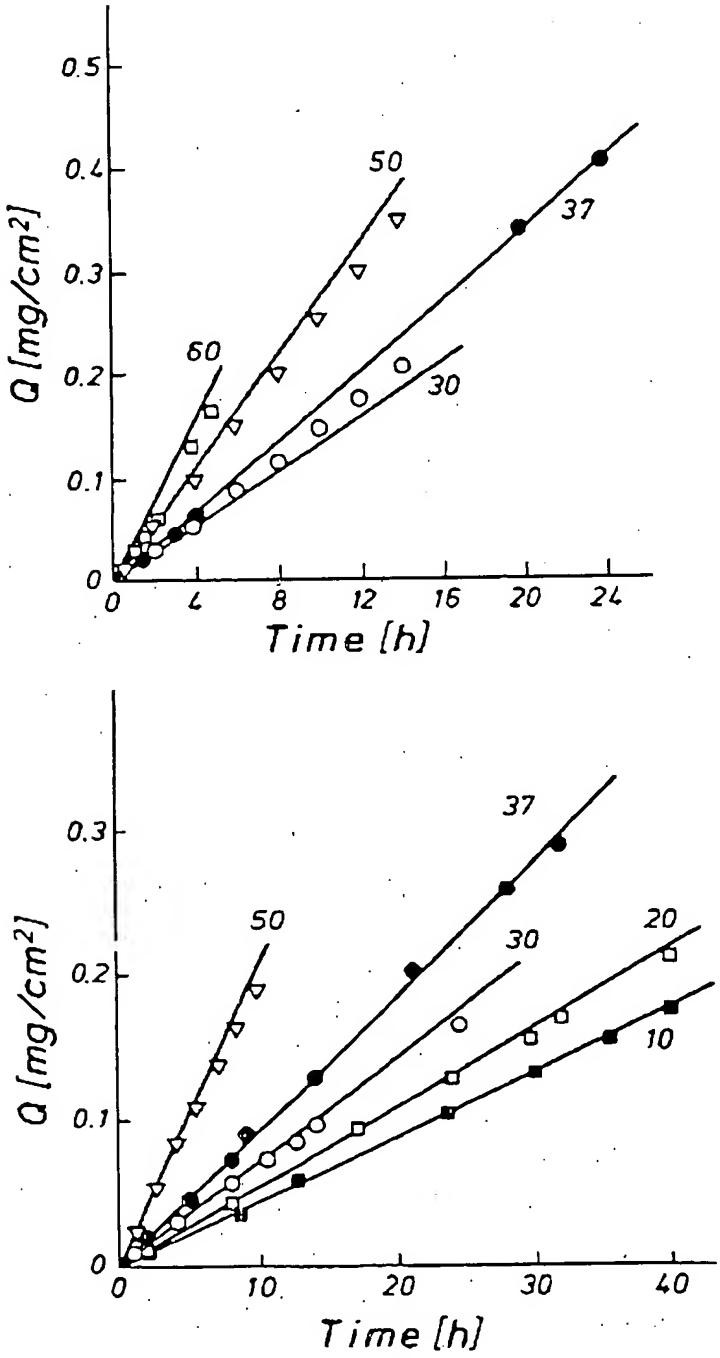
permeation results from both the symmetric as well as the asymmetric temperature designs. Thus, the diffusion coefficient change as a function of system temperature may be expressed as follows:

$$D = D_0 \exp\left[\frac{-E_d}{RT_0} \times \frac{T_0}{T - 1}\right] \qquad \text{(eq. 2)}$$

where D_0 is the drug diffusion coefficient in the membrane at T_0 (310.15°F = 37°C), R is the gas constant, and E_d is the activation energy for diffusion.

The temperature effect on skin permeation of nitroglycerin was recently studied in vitro using hairless mouse skin (Momii et al. 1995). The donor chamber temperature was set at 12, 22, 32, 37, and 42°C in each permeation

Figure 10.2. Permeation Profiles of Desoxycorticosterone (2a—upper figure) and Progesterone (2b—lower figure) Through Silicone Membrane



The different markers represent the experimental data from various donor temperatures as indicated by the numbers; the lines represent calculated values. Reproduced with permission from Tojo et al. (1987).

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experiment, while the receptor chamber temperature was maintained constant at 32°C. The experimental results are summarized in Table 10.1. The diffusion coefficient of nitroglycerin in the stratum corneum and viable skin agreed with the Arrhenius relationship with the activation energy of 69.1 kJ/mol and 46.1 kJ/mol, respectively. The partition coefficient remained virtually unchanged within the temperature range tested (12–42°C). These experimental results confirmed the validity of Equation 2, which predicts the concave curvature when a flux is plotted against system temperature with an unchanged partition coefficient.

The effect of environmental perturbations on percutaneous absorption of topical parathion through porcine skin was studied in vitro (Chang et al. 1994). The parameters investigated were air temperature, perfusate temperature, perfusate flow, and relative humidity. They found that increasing air temperature from 37 to 42°C, relative humidity from 60 percent to 90 percent, and doubling the perfusate flow, each produced a significant effect on parathion absorption. These findings may also be explained according to Equation 1: increasing air temperature might have increased the diffusion coefficient D and upstream concentration C_d; increasing relative humidity might have increased the stratum corneum hydration and thus might have also contributed to the increase of diffusion coefficient D; increasing the perfusate flow might have facilitated the removal of parathion from the skin tissue and, therefore, might have decreased the downstream permeant concentration C_r. All of these would result in an enhancement in the skin absorption of topically applied parathion.

Effect of Exercise and Temperature on In Vivo Drug Absorption

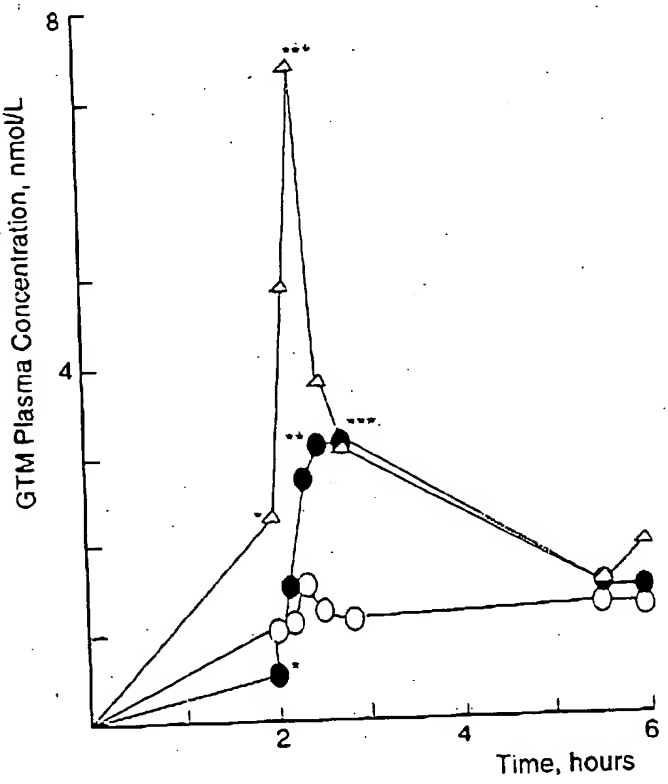
The effects of exercise and elevated ambient temperature on nitroglycerin skin absorption were studied using a commercial transdermal nitroglycerin device (Transderm-Nitro®) (Barkve et al. 1986). Two hours after a Transderm-Nitro® patch was applied to the chest, the change in plasma nitroglycerin concentration of 12 healthy volunteers was monitored during and after 20 minutes of exercise with a bicycle ergometer, and 20 minutes in a low-humidity, 90°C sauna, respectively. The bicycle ergometer exercise resulted in a threefold increase of plasma nitroglycerin concentration (i.e., from 1.0–1.5 nmol/L of control condition to 3.1 nmol/L), whereas the sauna caused about a sevenfold increase to 7.3 nmol/L (Figure 10.3). As a consequence of the dramatically increased plasma nitroglycerin concentration, 10 subjects reported side effects from the overdose of nitroglycerin during the study. Skin temperature measurement indicates that there was only a small increase during sauna treatment (up to 38.1°C). No temperature measurement was made on the temperature increase of the transdermal device. For the bicycle

Table 10.1. Effect of Temperature on Permeation Parameters

Parameter	Skin	Temperature of Donor Solution (°C)				
		12	22	32	37	42
dQ/dt ($\mu g/cm^2/hr$)	intact	3.23 + 0.43	9.98 + 1.81	20.39 + 1.08	29.39 + 3.50	49.35 + 3.19
	stripped	20.92 + 6.37	50.91 + 16.77	66.05 + 15.69	105.24 + 13.56	136.80 + 35.33
t_d (hr)	intact	3.02 + 0.57	1.48 + 0.23	0.56 + 0.08	0.59 + 0.12	0.30 + 0.06
	stripped	0.47 + 0.06	0.22 + 0.11	0.11 + 0.04	0.10 + 0.02	0.08 + 0.03
D (cm^2/s)	s.c.	3.44×10^{-11}	7.31×10^{-11}	2.52×10^{-10}	2.02×10^{-10}	6.79×10^{-10}
	v.s.	1.35×10^{-7}	2.77×10^{-7}	5.81×10^{-7}	6.53×10^{-7}	8.13×10^{-7}
K (–)	s.c.	385	596.14	406.68	700.87	394.43
	v.s.	19.9	22.43	14.59	20.69	21.63

Receptor solution: Saline solution containing 20% PEG 400. dQ/dt: Steady state rate of permeation. t_d: Time lag. D: Diffusion coefficient. K: Partition coefficient. The thickness of skin: whole thickness = 370 μm; stratum corneum = 10 μm. s.c. = stratum corneum. v.s. = viable skin. Reproduced with permission from Mornii et al. (1995).

Figure 10.3. Mean Plasma Concentrations of Glyceryl Trinitrate (GTN) (n = 12)



Control day (empty circle), exercise day (filled circle), and sauna day (empty triangle). Compared to control day: *p < 0.05; **p < 0.01; and ***p < 0.001. Reproduced with permission from Barkve et al. (1986).

ergometer exercise, there was a decrease in skin temperature due to sweating (down to 33.3°C). The investigators concluded that the skin temperature was not the sole determinant for the significantly enhanced skin absorption of the drug. They attributed the increased drug uptake primarily to vasodilation and increased blood circulation. It is possible that other factors also played an important role. For example, sweating might have accelerated the stratum corneum hydration, and thus, might have increased the drug diffusion coefficient D in the stratum corneum (Equation 1). Elevated temperature of the transdermal patch in the sauna treatment might have increased the drug solubility C_d, hence its concentration gradient and the driving force

for drug permeation. This may provide an explanation as to why the much higher plasma drug concentration in the sauna treatment was observed as opposed to the exercise treatment, since skin vasodilation was present in both cases.

Similar results were recently reported on the effect of physical exercise on plasma nicotine concentration in 8 healthy subjects treated with a nicotine transdermal patch (Klemsdal et al. 1995). After 11 hours of patch application, plasma nicotine concentrations were measured before and after 20 minutes of moderate bicycle exercise, or 20 minutes of rest. Mean plasma nicotine concentration increased from 9.8 to 11.0 ng/mL during exercise, as compared to the unchanged control during resting (from 10.5 to 10.2 ng/mL). Again, the increased skin absorption of nicotine was attributed to the exercise-induced increase in skin blood flow at the patch application site.

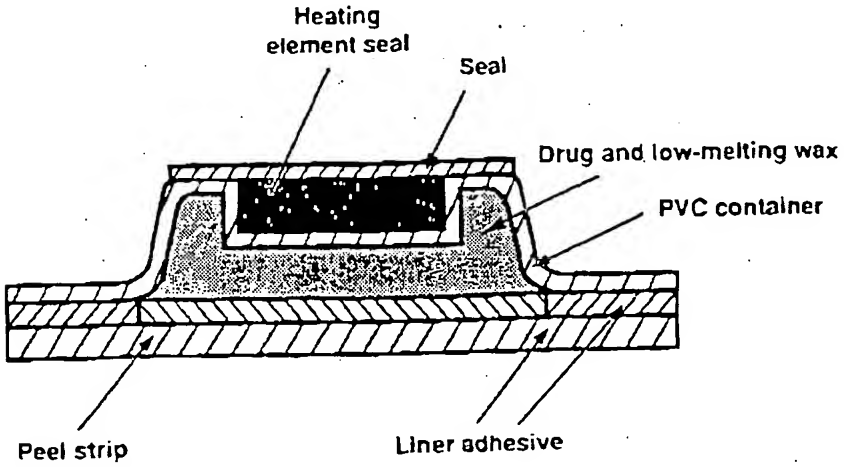
These in vitro and in vivo studies demonstrated that elevated surrounding temperature did cause increased skin permeation of the drugs, indicating the potential risk of drug toxicity by overdose. On the other hand, one may take advantage of the thermal energy-enhanced percutaneous absorption to increase topical drug delivery and TDD.

Use of Thermal Energy for Skin Absorption Enhancement

It is intuitive that applying heat to a skin area to which a topical drug has been applied would enhance drug absorption. Physiotherapists have a long history of combining topical drug applications with thermal treatment. In China, for example, it is a common practice to place a thermal pad over a plaster containing certain medicaments, such as local anesthetics, as an anecdotal way of enhancing the efficacy of the drug. The common superficial heating systems include an infrared lamp and infrared heating pad, the hydrocollator pack, a rubber heating bottle, an electric heating pad, and chemical packs. The chemical packs currently available are in flexible containers in which, by removing the container, a compartment is broken. The subsequent mixing of the ingredients causes exothermic chemical reactions, thus producing heat (Lehmann and De Lateur 1990b; Orenberg et al. 1986).

A patented transdermal device design makes use of thermal energy for the enhancement of TDD (Konno et al. 1987). Figure 10.4 shows the structure of the self-heating transdermal patch. When the seal at the back of the patch is removed to expose the iron powder in the heating chamber to the air and water, the resulting exothermic reaction provides the heat to soften the low-melting wax in the drug reservoir and to facilitate percutaneous drug absorption. Another U.S. patent described a similar approach (Argaud 1990).

Figure 10.4. A Self-Heating Transdermal Patch from U.S. Patent 4,685,911



Despite the importance of temperature effects on the percutaneous absorption of drugs, the small number of reported studies, particularly using the asymmetric temperature design to mimic a clinical situation, indicates that this area is still poorly understood, and its potential as a permeation-enhancing technique appears so far underappreciated.

PERMEATION ENHANCEMENT BY ULTRASONIC ENERGY

Phonophoresis (Sonophoresis)

Ultrasound is a form of acoustic vibration propagated in the form of longitudinal compression waves at frequencies beyond the human audible range (i.e., frequencies above 20 kHz). This form of energy is used extensively in medical diagnosis as well as in therapeutic applications, such as physiotherapy and sports medicine (Griffin 1982). Phonophoresis is defined as the movement of drugs through intact skin and underlying soft tissues under the influence of an ultrasonic perturbation. Clinical experiences have demonstrated that phonophoresis is a safe technique for enhancing drug administration and is effective in clinical applications when used with a proper frequency, power level, and duration. Some recent research efforts in this area have been directed to elucidate the mechanism of phonophoresis, and to explore its applications in peptide and protein drug delivery (McElnay

et al. 1993; Bommannan et al. 1992a,b; Simonin 1995; Mitragotri et al. 1995a,b).

A hypothesis was proposed by McElnay and coworkers (1993) that phonophoresis increases drug permeation through the skin by disordering the structured lipids in the stratum corneum. The in vivo experimental results obtained with 10 healthy volunteers treated with methyl nicotinate under phonophoresis appears to support this hypothesis. Phonophoresis of 3.0 MHz, 1.0 W/cm^2 continuous output resulted in increased percutaneous absorption of the drug, which existed for a period after the ultrasound treatment had ceased.

Bommannan et al. (1992b) showed strong experimental evidence to support the notion that ultrasound treatment disrupts the stratum corneum and increases the intercellular transport of drugs as a mechanism of phonophoresis. Using a colloidal tracer, lanthanum hydroxide, and high frequency ultrasound of 10 or 16 MHz in vivo on hairless guinea pig skin, they obtained electron micrograms showing that the tracer penetrated through the stratum corneum and the underlying viable epidermis via an apparently intercellular route (Bommannan et al. 1992b). It was demonstrated that the tracer was able to be transported through the epidermis to the upper dermis with remarkable speed of only 5 minutes of the ultrasound treatment. In a separate study using salicylic acid as a model drug (Bommannan et al. 1992a), the same group of researchers demonstrated the following:

1. Ultrasound treatment of high frequencies significantly increases the skin permeation of salicylic acid as compared to the passive diffusion control without phonophoresis.
2. Pretreatment of the skin with ultrasound lowered the skin barrier function such that the subsequent salicylic acid delivery was enhanced in comparison to the passive diffusion control.
3. Ultrasound did not alter the release kinetics of salicylic acid from the gel formulation used.

All of these results indicate that the skin absorption enhancement by phonophoresis is a direct effect of ultrasound on the stratum corneum.

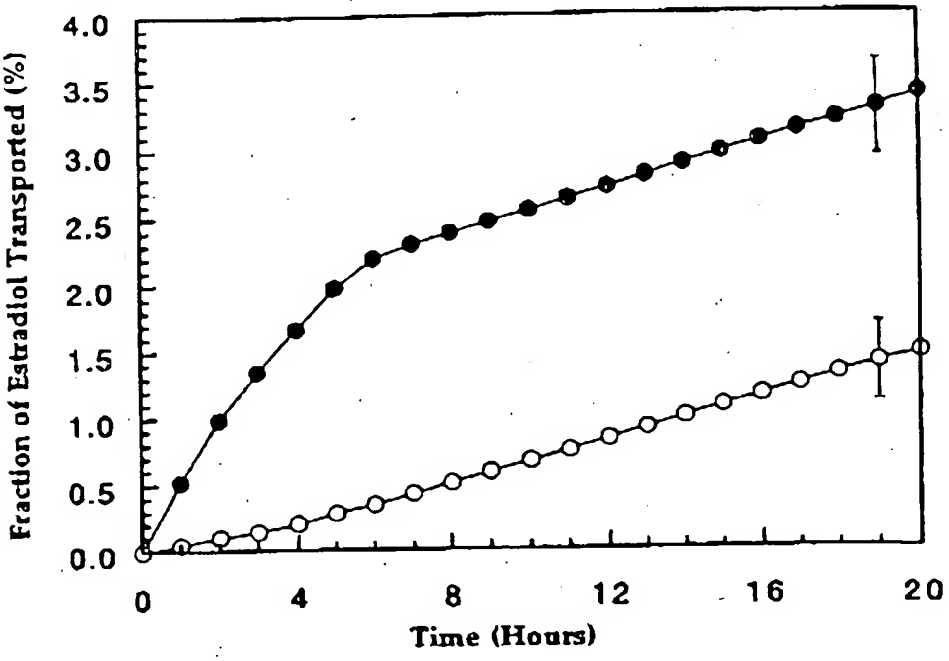
Simonin (1995) offered an in-depth critical review on the mechanism of phonophoresis. Several previously proposed mechanisms were examined, namely, the thermal effect, boundary layer reduction, acoustic pressure, decrease of the donor solution-membrane interfacial potential energy barrier, and cavitation. It was believed that although increased temperature would lead to enhanced skin permeability, the direct temperature effect on the diffusion coefficient was less than 20 percent due to the rather small increase

in skin temperature. However, the subsequent increase in drug solubility, skin vasodilation, and blood flow all contribute to enhanced percutaneous absorption. The most significant physical mechanism of phonophoresis is likely to be cavitation and microstreaming, which cause a vigorous mixing, thereby facilitating the drug diffusion process. For hydrophilic drugs, this acoustic energy–facilitated permeation process was proposed to take place in the follicular pathways, such as sweat glands and hair follicles.

A study was conducted to evaluate the relationship of skin permeation enhancement and various ultrasound-related phenomena, including cavitation, thermal effects, generation of convective velocities, and mechanical effects (Mitragotri et al. 1995b). The in vitro experiments were conducted using heat-separated human epidermis membrane. The ultrasound parameters used were within the therapeutic ultrasound range (i.e., frequency: 1–3 MHz and intensity: 0–2 W/cm^2). The results indicated that among all the ultrasound effects evaluated, cavitation appeared to play the dominant role in ultrasound-facilitated TDD. Figure 10.5 shows that estradiol permeation was enhanced by the application of ultrasound as compared to passive diffusion control. It is clear from the graph that drug permeation enhancement by ultrasound followed a time-dependent reduction (i.e., initially high) then gradually diminished over a period of 6 hours. It was hypothesized that the observed reduction in estradiol permeation was due to the degassing of the liquid surrounding the skin by the continuous ultrasound treatment. Dissolved air is necessary for cavitation. If cavitation was an important condition for phonophoresis, weakened cavitation by degassing the medium would reduce the permeation enhancement. The experimental conditions were modified to verify the hypothesis. During this permeation experiment, both donor and receptor media were aerated every hour by bubbling air through them for 5 minutes. The results in Figure 10.6 confirmed the importance of air content in the medium: drug permeation in the aerated medium remained high, in contrast to the leveling off in the solution not aerated. Another interesting observation made by the same group of investigators was that a 30-minute ultrasound treatment (1 MHz, 2 W/cm^2) to a piece of skin, that had been soaked in a fluorescence solution for 5 days caused the bleaching of fluorescence in the keratinocytes, but not fluorescence in the intercellular lipids. The bleaching of fluorescence was attributed to the possible formation of hydrogen peroxide generated by the applied ultrasound. It was, therefore, concluded that ultrasound induces cavitation in the keratinocytes.

Ultrasound-mediated transdermal delivery of protein drugs was recently reported (Mitragotri et al. 1995a). In the in vitro experiments, low-frequency ultrasound (20 kHz, 100 ms pulses applied every second) was applied to the heat-separated human epidermis from the side of donor solution with the

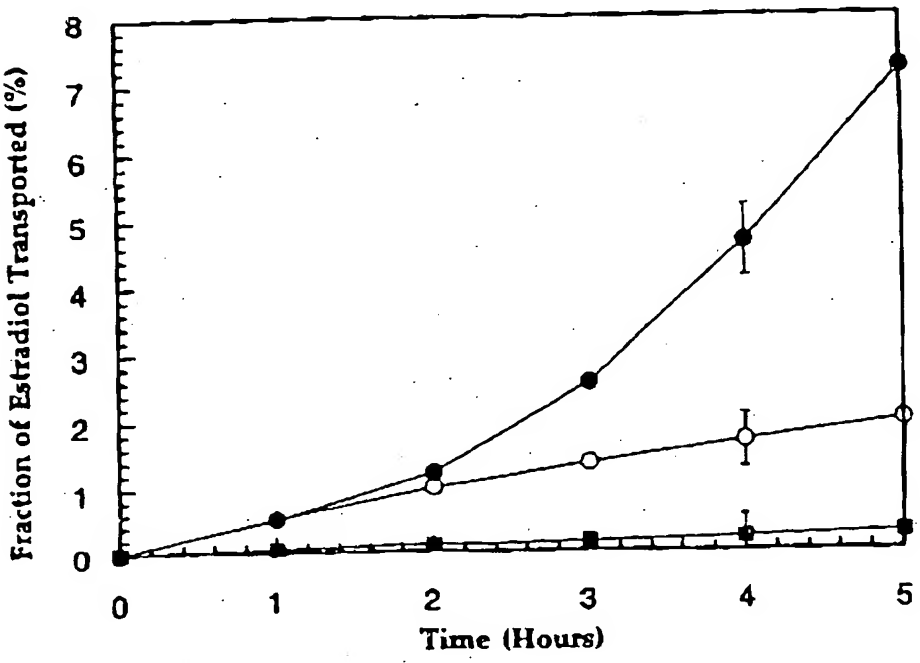
Figure 10.5. Variation of the Transdermal Estradiol Flux with Time During Ultrasound Exposure



(1 MHz, 2 W/cm^2). Key: filled circle, in the presence of ultrasound; empty circle, in the absence of ultrasound (passive controls). Typical error bars (SD) are shown on one data point. Reproduced with permission from Mitragotri et al. (1995b).

intensities in the ranges of 12.5–225 mW/cm^2 for 4 hours. Three protein drugs evaluated were insulin (MW ~ 6,000), interferon gamma (IFN-Γ, ~ 17,000) and erythropoietin (MW ~ 48,000). Ultrasound application induced significant transdermal permeation of all the protein drugs tested. The permeability of heat-separated human epidermis to insulin with ultrasound intensity 225 mW/cm^2 is 3.3×10^{-3} cm/hr, to IFN-Γ 8×10^{-4} cm/hr, and to erythropoietin 9.8×10^{-6} cm/hr. Figure 10.7 shows the in vitro permeation profiles of insulin at various ultrasound intensities. It should be noted that transdermal administration of therapeutic quantities of protein drugs would be achieved by ultrasound-facilitated transdermal delivery if the in vitro results with human epidermis membrane can be reproduced in vivo in humans. The results from this in vivo animal experiments appear promising. The use of low-frequency ultrasound to deliver insulin into the skin of diabetic hairless rats effectively corrected hyperglycemia.

Figure 10.6. Effect of Repeated Aerating on the Transdermal Flux of Estradiol in the Presence of Ultrasound

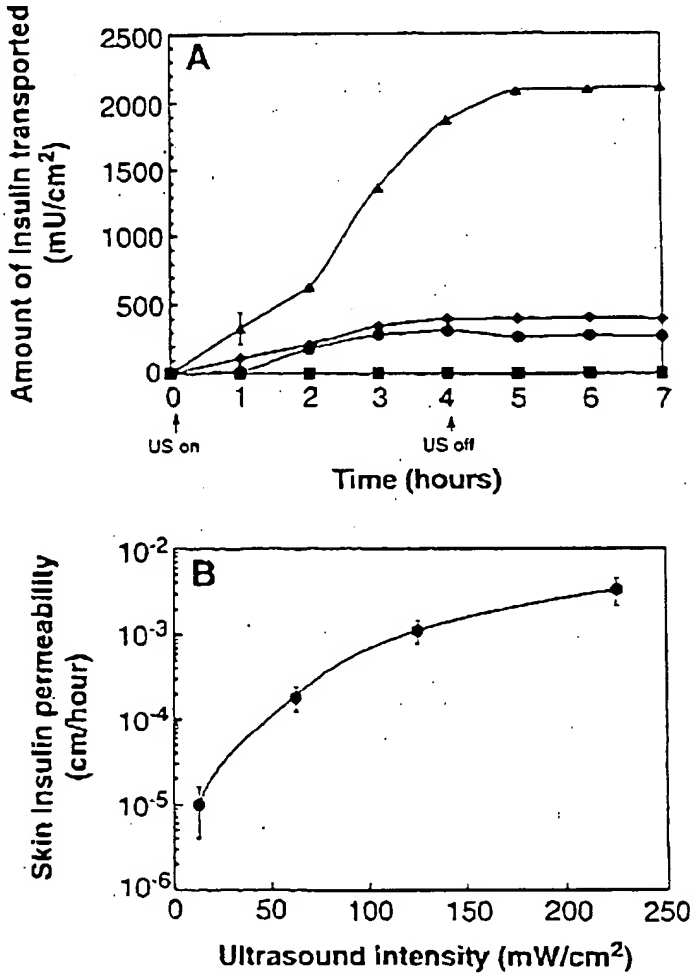


(1 MHz, 2 W/cm²). Key: filled circle, donor solution aerated every hour; empty circle, no repeated aerating; filled square, passive controls. Typical error bars (SD) are shown on one data point. Reproduced with permission from Mitragotri et al. (1995b).

Effect of Ultrasound Frequency on Phonophoresis

Given the profound implication that protein drugs with molecular size up to 48,000 daltons can be delivered transdermally in therapeutic quantities, the effect of ultrasound frequencies on phonophoresis clearly merits a thorough examination. Most medical applications use ultrasonic frequencies between 0.75 MHz and 15 MHz. For example, physiotherapy uses 1–3 MHz, diagnostic 1–10 MHz, and general ultrasonic 4–8 MHz (Sun and Liu 1994). It is known that the penetration ability of ultrasound into soft tissues is inversely proportional to its frequency (e.g., at 0.09 MHz, approximately 50 percent of acoustic energy penetrates to 10 cm depth; with 1 MHz, 5 cm; and with 4 MHz, only to 1 cm). Griffin and Touchstone (1972) studied the effect of ultrasound frequency on phonophoretic delivery of hydrocortisone into pig skin in vivo (1 W/cm², 17 min). Among five different frequencies

Figure 10.7. Insulin at Various Ultrasound Intensities



(A) Time variation of the amount of insulin transported across human skin (in vitro) in the presence of ultrasound (20 kHz, 100 ms pulses applied every second) at 12.5 (filled square), 62.5 (filled diamond), 125 (filled circle), and 225 mW/cm² (filled triangle) (n = 3 or 4; error bars, SD). (B) Variation of the transdermal insulin (in vitro) with ultrasound intensity (20 kHz, 100 ms pulses applied every second) (n = 3 or 4; error bars, SD). The skin is impermeable to insulin at an ultrasound intensity of 0. Reproduced with permission from Mitragotri et al. (1995a).

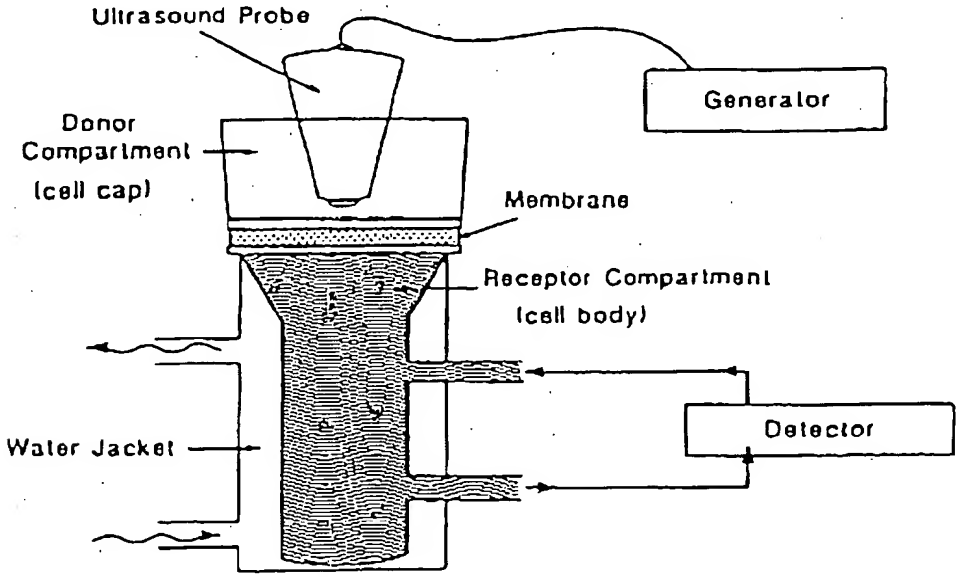
investigated (i.e., 0.09, 0.25, 0.5, 1.0, and 3.6 MHz), 0.25 MHz ultrasound yielded the highest mean hydrocortisone concentration in tissues, and 3.6 MHz yielded the second highest value, whereas 1.0 MHz, the frequency

used most commonly in physiotherapy, delivered the least amount of hydrocortisone. An uncomfortable sensation was found to be associated with 0.25 MHz ultrasound with the human subjects tested, which led the investigators to choose the 3.6 MHz frequency as the preferable frequency for minimal possibility of causing skin damage. Because the ultrasound intensity and duration of application were quite different between the studies by Mitragotri et al. (1995a) (225 mW/cm^2, 4 hours) and Griffin and Touchstone (1972) (1 W/cm^2, 17 minutes), direct comparison could not be made. The potential hazards associated with dental ultrasonic descalers, which generally operate at frequencies of 25–42 kHz, were examined (Walmsley 1988). Acoustic microstreaming and large shear forces resulting from the low-frequency ultrasound were reported to rupture erythrocytes and platelets both in vitro and in vivo (Walmsley et al. 1987). This resulted in activation of the blood coagulation system with subsequent thrombus formation. A thorough safety evaluation on the use of low frequency ultrasound for skin absorption enhancement is needed.

Mitragotri et al. (1995a) used the low frequency ultrasound in their work based on the hypothesis that if cavitation plays an important role in ultrasound-facilitated skin permeation, and since the cavitation threshold (i.e., the minimal ultrasound intensity required to cause cavitation) increases rapidly with an increase of ultrasound frequency, lower frequency would cause more cavitation and hence, higher skin permeation of a drug. On the other hand, Bommannan et al. (1992a) selected 16 MHz ultrasound based on the hypothesis that higher frequency ultrasound would better localize the acoustic energy within the stratum corneum and, therefore, would provide more perturbation in the skin barrier layer to facilitate drug permeation. The permeation results appear to support this hypothesis since both the extent and the rate of absorption of salicylic acid were enhanced with 10 and 16 MHz ultrasound treatment in comparison to 2 MHz. The most commonly used ultrasound frequency in physiotherapy and sports medicine is 1 MHz. This frequency was deliberately chosen as a compromise frequency between those that produce predominantly thermal effects (2 MHz and higher) and those that produce nonthermal (mechanical and/or chemical) as well as thermal effects (500 kHz or lower) in human soft tissues (Griffin and Karselis 1988). It is possible that the skin permeation enhancement observed with low frequency ultrasound and high frequency ultrasound is through two different mechanisms. This subject clearly merits further investigation.

A typical experimental setup for in vitro phonophoresis is shown in Figure 10.8. The ultrasound probe is a transducer that converts high frequency alternating voltage into acoustic vibrations using a piezoelectric crystal.

Figure 10.8. A Schematic Diagram of a Typical Experimental Setup for In Vitro Phonophoresis Study



Reproduced with permission from Kost et al. (1991).

Contacting the ultrasound probe with the drug solution in the donor compartment provides the pathway for the ultrasonic energy to reach and to perturb the skin membrane. In vivo experiments are usually conducted in a similar way, except that the pulsed ultrasound (i.e., discontinuous waveforms) and/or moving sound head techniques are often used to reduce the ultrasound dose for minimal tissue damage.

PERMEATION ENHANCEMENT BY ELECTRIC ENERGY

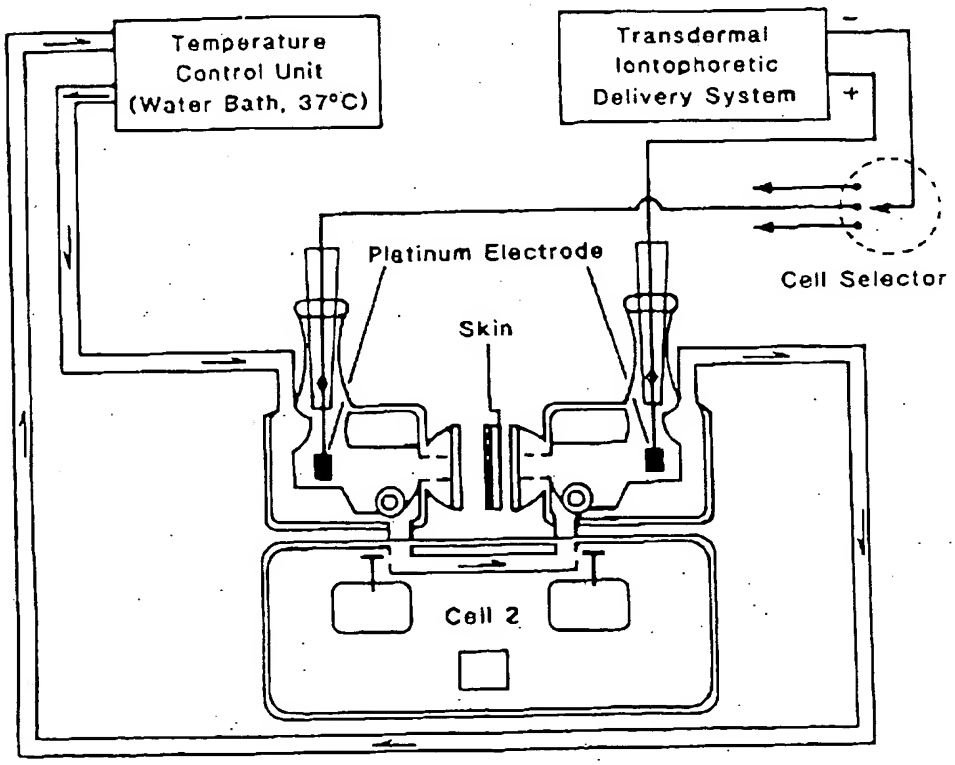
Iontophoresis

Using electric energy to deliver drugs into the skin for medical treatment has a very long history. According to a comprehensive review on the history of electric therapy (Licht 1967), the first attempt to administer a medicine (an antiapoplectic drug) into human skin in vivo by electricity was made in 1747 using a Leyden jar, the simplest and earliest form of a capacitor for storing electric charges. In 1833, 51 years before the theory of electric dissociation was published by Arrhenius, Fabre-Plaprat used a direct current to administer quinine to cure quartan fever and iodine to treat sarcocele. Since then,

iontophoresis has found some uses for the administration of drugs through the skin in the clinics of certain disciplines of medicine, primarily in physiotherapy, sports medicine (Harris 1967; Griffin and Karselis 1988), and dermatology (Sloan and Soltani 1986). It was the progress made, or rather, the obstacles encountered, in TDD for systemic treatment in recent years that revived interest in this technology. As it became clear that most drugs cannot permeate through human skin in therapeutic quantities by passive diffusion alone, and almost all peptide and protein drugs cannot permeate into the skin at all because of their large molecular size and hydrophilicity, the need for permeation enhancement techniques brought iontophoresis research to the front line.

A typical experimental setup for in vitro iontophoresis is shown in Figure 10.9. A piece of skin is mounted between the two compartments of side-by-side diffusion cells. Two conductive electrodes are immersed in the donor

Figure 10.9. A Schematic Diagram of a Typical Experimental Setup for In Vitro Iontophoresis Study



Reproduced with permission from Chien et al. (1988).

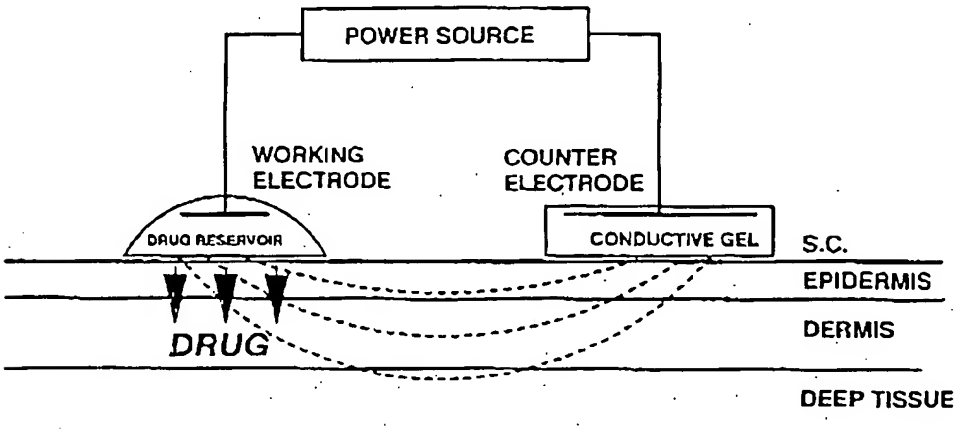
and receptor solutions. The power source is connected to the electrodes by proper polarities, according to the charge carried by the ionic drug species to be delivered. The electrodes are usually made of noble metals, such as platinum, or composed of silver coated with its halide salts, such silver chloride or silver bromide. The use of Ag/AgCl or Ag/AgBr electrodes has an advantage over the platinum electrode in that the electrolysis of water is prevented, as a consequence, the solution pH will not shift. A typical in vivo iontophoresis system is shown in Figure 10.10.

In terms of the experimental setup, electro-osmosis is almost identical to that for iontophoresis. When delivery of a nonionic drug is of concern, the donor compartment is charged with the drug solution, and the solution pH and electric polarity of the electrodes are arranged in such a way that the applied electric field results in an inward movement of the fluid into the skin. If the sampling of biosubstances is the objective, the arrangement is reversed. The content of the "donor" compartment is analyzed for the biosubstance carried out by the outward movement of the fluid, being either "receptor" fluid in in vitro experiments, or interstitial fluid in in vivo studies. In electroporation studies, the basic experimental setup remains the same while the output of the power source changes to high voltage and short pulses.

Electroporation

Electroporation is a phenomenon in which the membrane of a cell exposed to high-intensity electric field pulses (up to several hundred volts for

Figure 10.10. A Schematic Diagram of a Typical Experimental Setup for In Vivo Iontophoresis Study



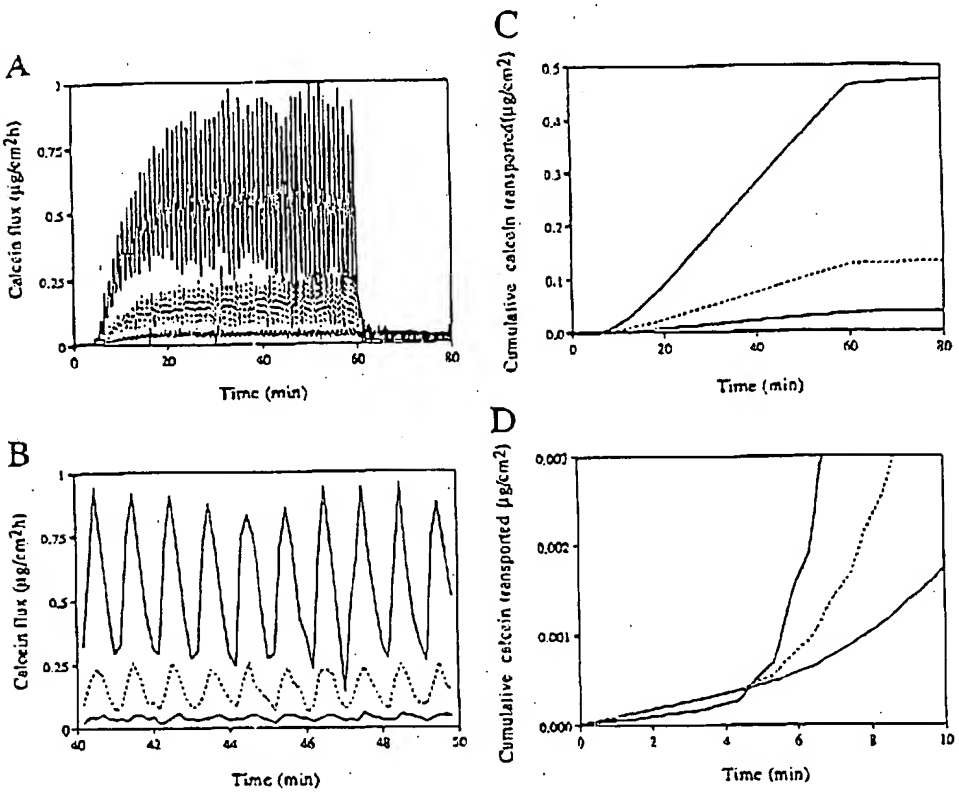
Reproduced with permission from Liu and Sun (1994).

micro- or milliseconds) can be temporarily destablized, thus becoming highly permeable to exogenous molecules in the surrounding media. Electroporation may be regarded as a microinjection technique and, therefore, has been used extensively in delivering test materials into isolated cells in vitro (Chang et al. 1992) and, occasionally, into living cells in vivo (Titomirov et al. 1991). A research team at MIT (Weaver et al. 1989; Prausnitz et al. 1993) was the first to demonstrate that electroporation increased drug permeation through the skin. Working with heat-separated human epidermal membrane in vitro, and hairless rat skin in vivo, they reported a flux increase up to 4 orders of magnitude for 3 polar model compounds with charges between -1 and -4 and molecular weight slightly over 1000 daltons. The electroporation conditions used were as follows: an exponential-decay pulse (exponential-decay time constant, $\tau = 1.0–1.3$ msec) was applied every 5 seconds for 1 hour; the electric voltage ranged from 0 to 500 volts. It was found that a transition point might exist at about 100 V, below which flux increase was reversible (i.e., skin barrier function recovered after cessation of electroporation), and above which the effects were only partially reversible. The effect of electroporation voltage on the permeation of calcein (623 daltons, -4 charge) through the human epidermis membrane is shown in Figures 10.11a–d (Prausnitz et al. 1994). These figures clearly show characteristically rapid response of permeant flux to the electric pulse (i.e., in seconds to minutes). A recent report from the same research group (Edwards et al. 1994) provided a theoretical analysis of electroporation for TDD under two electroporation conditions (i.e., at small and large transdermal voltages). In this model, charged molecules were considered transporting through existing shunt routes of the skin at transdermal voltage $\leq$ 100 V. When transdermal voltage was greater than 100 V, the transcorneocyte pathway was also accessible to charged molecules as lipid bilayers were electroporated. The available experimental results compared favorably with the respective theoretical predictions in the respective small and large electrical field strengths.

Reverse Iontophoresis and Noninvasive Diagnostic Applications

In another study conducted by Fabre-Plaprat in the early 17th century (Licht 1967), hydroiodate was placed on the skin of one arm, and a starch solution on the other. Upon application of electric current, Fabre-Plaprat claimed that iodide was delivered into the body, reached the skin of the other arm, and reacted with the starch. This was probably the first attempt on noninvasive sampling of chemical substances from the skin by electricity, now known as reverse iontophoresis sampling. Electrically administered iodide was later recovered in both the urine and saliva by another scientist in a separate study.

Figure 10.11. Time Profiles of Transdermal Transport of Calcein Due to Electroporation at Different Voltages



(A) Transdermal flux due to pulsing at 1 pulse per minute (ppm) for 1 hr, 270 V (solid line), 135 V (dashed line), 115 V (dotted line). (B) Data in (A) with the time axis expanded. (C) Data in (A) replotted as cumulative calcein transported, from calculation of steady state lag times, indicated by the time-axis intercept. (D) Data in (C) with time axis expanded, to show transport onset time. Reproduced with permission from Prausnitz et al. (1994).

The use of reverse iontophoresis for noninvasive sampling of biosubstances is an interesting, and potentially very important, application of the electro-osmosis technique. Benjamin et al. (1954) described the use of reverse iontophoresis to obtain interstitial fluid in a noninvasive manner for the analysis of tissue electrolytes. With a device setup similar to that shown in Figure 10.10, the authors conducted reverse iontophoresis tests on 98 human subjects, and subsequently analyzed the amounts of sodium and potassium

extracted from the skin. The results were reproducible. The application of reverse iontophoresis for noninvasive glucose monitoring was recently investigated with promising results (Heil and Kadera 1989; Glikfeld et al. 1989; Rao et al. 1993; Glikfeld et al. 1994). If reverse iontophoresis can serve as a means of noninvasive glucose monitoring with good reproducibility, it will have a tremendous impact on diabetes management.

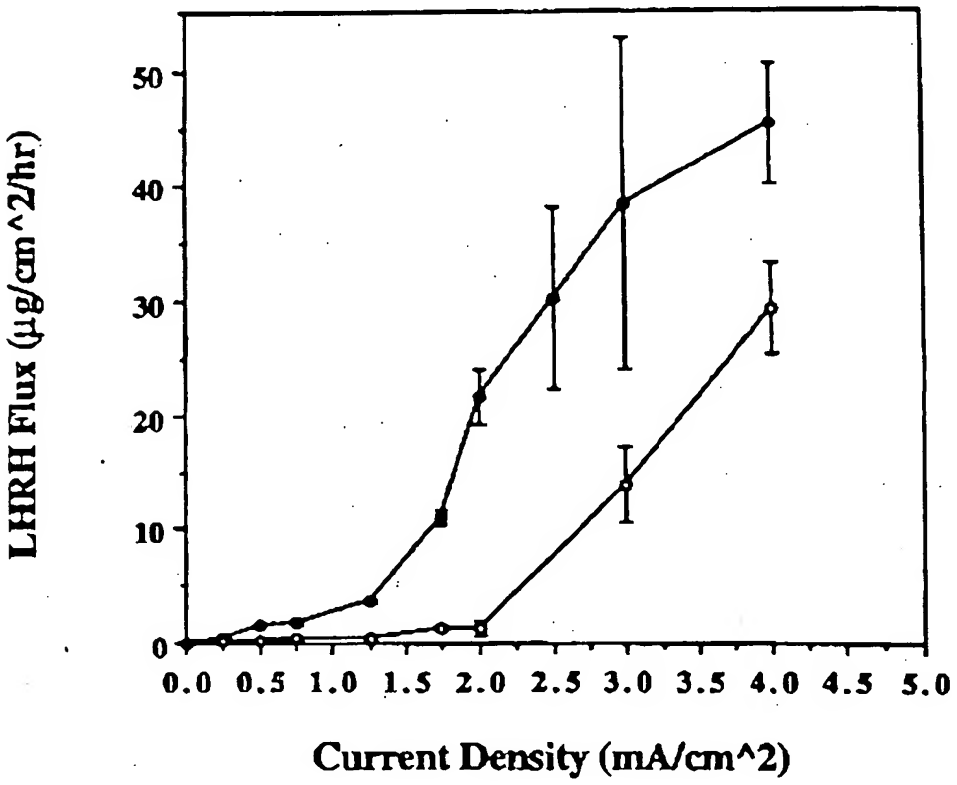
COMBINATION OF DIFFERENT ENHANCEMENT METHODS

Combining different enhancement methods can further improve drug permeation through the skin. A recent study (Bommannan et al. 1994) reported the use of electroporation in combination with iontophoresis for the transdermal delivery of luteinizing hormone release hormone (LHRH, 1182 daltons). The experiments were conducted in vitro using human epidermis membranes. Comparisons were made between iontophoretic LHRH flux obtained at current intensity ranging from 0 to 4 mA/cm^2, with and without electroporative electric pulse (1000 V, $\tau = 5$ msec). The results indicated that the application of a single electroporative pulse prior to 30-minute iontophoresis consistently yielded 5–10 times higher flux than iontophoresis alone (Figure 10.12). The LHRH flux at two hours after iontophoresis decreased to a value significantly less than the maximal value obtained during iontophoresis, approaching the pretreatment value. These results suggest that a combination of electroporation and iontophoresis, with the former transiently altering the skin permeability to the drug, and the latter providing the primary driving force to the permeation process, would allow the enhanced delivery of peptides that cannot be effectively delivered by other transdermal means. Kost et al. (1996) showed that the combination of ultrasound and electric field brought about a synergistic effect on the transdermal transport of two model compounds, calcein and sulphorhodamine. As shown in Figure 10.13, much higher sulphorhodamine flux through heat-separated human epidermis was obtained during simultaneous application of ultrasound and electric field than the presence of electric field alone.

FUTURE DIRECTIONS

More work must be done to elucidate the underlying mechanisms for the methods of physical skin permeation enhancement, especially for phonophoresis and electroporation. The combination of different enhancement methods for an additive or synergistic effect is a logical expansion of current research activity in this field. Carefully designed experiments must

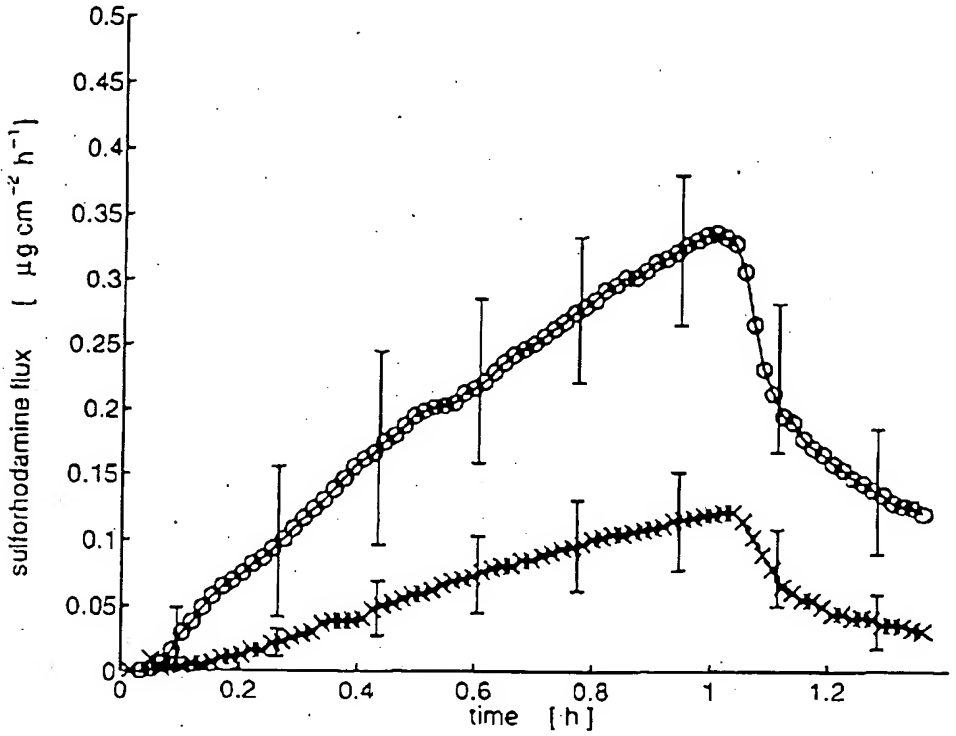
Figure 10.12. Iontophoretic Flux of LHRH (mean ± SD)



Shows nonlinear increase with current density with (filled circle) and without (empty circle) a pulse. $p \leq 0.01$ for all current densities (except 3.0 mA/cm^2, $p < 0.2$) when flux with and without pulse are compared. Reproduced with permission from Bommannan et al. (1994).

be conducted to examine the possible side effects associated with these methods, and to define a safe, yet effective, range in utilizing these energy forms for percutaneous drug delivery. Since most of the clinical evidence showing the effectiveness of the physical enhancement methods was from case studies, well-designed clinical investigations with proper controls are clearly needed to provide the documentation required for any products based on the technologies. Finally, it is a challenge to the drug delivery scientist to invent the ultimate TDD system that is effective, safe, user-friendly, cost-effective, as well as powerfully versatile (e.g., microchip controlled for complex drug delivery patterns).

Figure 10.13. Time Variation of Sulphorhodamine Flux



In the presence of electric field alone (X) and during simultaneous application of ultrasound and electric field (O). Ultrasound was ON all the time (O). Electric voltage was turned ON at time 0 and was OFF at 1 hour in both cases (O as well as X). Presented as means and SD of at least three repetitions. Reproduced with permission from Kost et al. (1996).

SUMMARY

Passive topical and transdermal drug delivery has been the generally recognized convention for the local or systemic administration of drugs, respectively, for many decades. New groundbreaking technologies have been developed for the enhancement of drug delivery by the use of various physical means. Variations in skin temperature have been shown to correlate with regional variations in percutaneous drug absorption. The advantage of this fact has led to enhanced drug delivery systems utilizing thermal energy in the form of local heat applied to the skin to facilitate drug absorption. Ultrasonic energy delivered from devices is being used to enhance therapeutic

efficacy in the treatment of local conditions by a process of phonophoresis and sonophoresis. More recently, new technologies and devices have been developed, whereby drug permeation enhancement for transdermal administration is being employed for the treatment of systemic diseases. These forms of electrically assisted drug delivery systems (i.e., iontophoresis and electroporation) alone or in combination with chemical permeation enhancers may provide new possibilities for the transdermal delivery of drugs that could not be delivered by passive means in the past.

ACKNOWLEDGMENT

The author is grateful to Ms. Elisabeth Kimbleton for her invaluable contribution to the preparation of this manuscript.

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